63. CONTROL AND ESTIMATION OF FUNGAL RESISTANCE OF LEATHER

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INTRODUCTION

Although molds have undoubtedly been growing on leather since it was first produced, surprisingly little attention was paid to the problem, until around 1945. Wilson (132) does not include either "mold" or "mildew" in the index; in 1928 (133) he treated the subject very sketchily, concerning himself mostly with microbial spoilage during tanning operations. McLaughlin and Theis (82) devoted less than one-half page to molds.

In his later work Wilson (134) gave an adequate description of molds, their physiology and methods of study, but he devoted less than one page to their control. Lollar and Steinle, in "Deterioration of Materials" (74), have presented the best treatment of the fungal resistance of leather that is found in any book published to date. Robertson in "Progress in Leather Science: 1920–1945" (99) discussed control of molds for about five pages.

At the outbreak of World War II the problem of mold growth on leather had received limited attention in the tropics. From the experience of Americans and Europeans in such places as the Philippine Islands, Sumatra, Malaya and Panama, it was well known that molds could attack a wide variety of materials, including the lenses of such optical in-

struments as microscopes and binoculars. That mold growth on materials in the tropics could pose very serious problems was well known to relatively few people. Those who were confronted with the problem used obvious means of dealing with the condition, such as burning lights in closets to decrease the humidity and keeping optical instruments in desiccators. The problems that would arise when thousands of men and their supplies were suddenly placed in the tropics were apparently realized by very few. At any rate, almost nothing had been done to combat the effects of a tropical climate on men and materials. As late as September 1944, only one tentative specification AXS 1416 (120) was available for protecting leather against molds (118). The experience of fighting a war in the tropics has served to focus attention on the tremendous losses that incur annually, due directly to deterioration of materials. Greathouse and Wessel (45), after studying the estimates of others, arrived at a conservative figure of 12 billion dollars per year for losses in the United States from all forms of deterioration, excluding foodstuffs. Leather goods undoubtedly comprise a significant part of this total. Turrentine (116) conservatively estimated the loss from mildew at 100 million dollars per year.

Leather, especially vegetable-tanned leather, is extremely susceptible to mold growth. Shoes, jackets, belts and instrument cases, in fact almost any leather item will develop mold growth almost overnight if subjected to the warm, moist conditions of the tropics. In most parts of the United States, leather goods are subjected to the growth of molds. Constant care must be taken to keep such goods dry and well aerated. The deterioration of leather goods used alone would be serious enough, but frequently leather is used in conjunction with or in the fabrication of other items. The effect of mold growth on some items may be serious enough to make them virtually useless. Among such equipment are optical instruments (55, 60, 122), medical supplies (57) and oxygen masks (104).

When large-scale fighting broke out in the South Pacific, the situation became critical. The writer was approached and told that even a 10 per cent improvement would justify the expense and trouble of treating the equipment. The seriousness of the situation can be seen when it is realized that shoes, for instance, would last only a few days (75, 126).

Some, perhaps most, of this work reported here, has been published but a great deal is buried in reports and memoranda. An attempt has been made to include as much of this material as possible in this chapter. Most of these reports are available at the National Research Council, Prevention of Deterioration Center, Washington, D. C.

EFFECTS OF MOLDS ON LEATHER

General Considerations

Although reports of leather articles rotting and falling apart have been made, especially from the tropical battlefields, a more careful and critical examination often failed to support these conclusions. Australian workers (7, 75) found that by far the most important cause of boots deteriorating in New Guinea was the use of iron components such as cutlan bills, heel attachment nails, lasting tacks, and toe and heel plates in their construction. Rusting of the ferrous grindery in itself weakens the boots; but, more important, the iron compounds combine with the leather and produce a material that no longer has the properties of leather. The leather-iron compound is black or bluish black, brittle, and has no resistance to wear. Mold growth contributes to the deterioration by increasing the humidity and preventing the leather from drying (102). Another type of failure of leather goods was the rotting of stitching thread, and in serious cases the article fell apart (1, 44). Direct effects of mold growth include damage to the grain of the leather and stains that cannot be removed without damaging the grain (1, 79).

Several attempts have been made to determine if molds actually damage the leather fibers. In 1943, Australian workers (7) reported studies in which vegetable-tanned leather was subjected to the growth of molds, then conditioned, and then the leather was tested for the breaking load and elongation at break. They were unable to demonstrate any significant loss of strength or change in elongation. Kanagy and co-workers (64) subjected vegetable-tanned strap leather to mold growth in a humidity cabinet, in a tropical room, and to conditions of soil burial. They could not demonstrate any breakdown of the basic chemical structure of the substance. There was a loss in tensile strength of 5 per cent, a loss in stretch at the breaking point, and an increase in stiffness. These slight changes in physical properties, Kanagy and others attributed to the breakdown of the oils and greases. These same conclusions were reached after further studies (66) in the same laboratory. Musgrave and Mitton (86) studied the effects of mold growth on the physical properties of catechol-tanned leather. After 6 months under conditions highly favorable to mold growth, very little change in the physical properties of the leather could be demonstrated. Some increase in stiffness and about a 7 per cent loss in strength occurred. This study confirmed that the molds utilize the water solubles as nutrients and that both mold growth and changes in physical properties are influenced by the oils in the leather. A study of the effect of mold growth on pyrogallol-tanned leather

by the same laboratory (56) gave essentially the same results as with catechol-tanned leather. Mold grew freely, but it caused very little damage. In a similar study on chrome-tanned leather, the same group of workers (84) obtained essentially the same results as with the vegetable tannages. A mold species, *Penicillium islandicum*, Sopp, not previously reported on leather, produced red stains when grown on leather samples containing mineral oil. Very little change in the physical properties of the leather occurred when the mold grew on it and the tensile strength was entirely unaffected. Day (32) exposed to the growth of fungi leather samples placed over moist gravel in covered pans. Loss in tensile strength after 30 days was not consistent nor significant. Martin (78) inoculated leather samples from various items of athletic equipment with an enriched soil suspension and determined breaking strength and stitch tear after molds had grown for 2 weeks. He found as many nominal increases in breaking strength as decreases, and more nominal increases in the stitch tear tests than decreases.

In a histological study, Barghoorn (9) examined moldy leather specimens after 2 and 6 months growth. Only minor invasion of fungus hyphae was observed in hair follicles and in the larger interstitial spaces between collagen aggregates. There was no evidence of the deterioration of the leather fiber matrix.

To summarize, fungi do not attack the leather fibers to any discernible extent. The stiffening and slight loss in strength that is sometimes found can be easily accounted for by the destruction of the fatty-type greases (64, 66, 69, 131), the destruction of tannin (7, 79), and the loss of glucose and other nontannins (1, 102) by the action of the molds.

Relation of Mold Growth to Other Types of Deterioration

In view of the evidence that fungi do not destroy leather substance, some explanation must be put forth for some of the earlier studies, such as those of Evans and Critchfield (38) and Colin-Russ (18), who reported a very definite loss of strength due to mold growth. In 1949, shoes that had been in storage on the island of Guam for about 5 years were examined (103). Mold growth was present but not on all shoes, nor were those showing mold growth completely covered. The leather of many of these shoes had deteriorated to such an extent that it could easily be torn by hand. There was, however, no evident relation between mold growth and deterioration. In some instances where a profuse growth had occurred there was no detectable loss of strength; in others, there was much loss of strength in the absence of mold growth. Sachs (103), after a study of the problem, concluded that "the most serious factor causing the deterioration of Marine Corps field shoes in

storage is continuous subjection to high relative humidities. This dele-
terious influence is assisted primarily by moderately high varying tem-
peratures and unsatisfactory pH (acidity) values of the leather initially.
Mildew growth present has no direct effect upon the hide substance of
the leather, but its function in removing the greases makes the leather
more vulnerable to the effects of high humidity." Kanagy and co-workers
(66) reached essentially the same conclusions, on the basis of a labora-
tory study in which leather samples were exposed at a temperature of
28° to 30°C and 95 to 100 per cent relative humidity. Leathers that
originally contained fatty oils and greases and were not protected with
a fungicide showed extensive losses of these fatty materials on ex-
posure to conditions favorable to mold growth. A decrease in the sa-
ponification numbers of the greases indicated that the fungi had hy-
drolyzed them. The deterioration as exhibited by loss in tensile strength
was attributed to high temperature and high relative humidity, which
brought about hydrolytic action on collagen. The extent of hydrolysis
depended upon the pH of the leathers. It increased slowly with in-
creasing acidity between pH 5 and 3; the action was much more rapid
below pH 3. Kanagy and co-workers also studied the effects of certain
treatments on the loss of tensile strength of seven different kinds of
leather. The results are presented in Table 1.

An examination of these data shows that no single treatment de-
creased the rate of degradation of all the leathers listed, although some
treatments have a beneficial effect on some leathers. Of particular in-
terest is the fact that the addition of sodium bicarbonate is beneficial

TABLE 1. LOSS IN TENSILE STRENGTH (LB PER SQ IN.) PER MONTH
OF EXPOSURE IN HUMIDITY CABINET OF 7 KINDS OF LEATHER
[FROM KANAGY AND OTHERS (66)]

			LEATHER				
Treatment	Sole	Chrome-Retanned Upper	Lace Indian Tan	Lace Raw Hide	Belting	Vegetable-Tanned Calfskin	Hydraulic Packing
Untreated	75	28	193	229	101	120	276
Fungicide	104	31	115	214	69	14	29
Degreased	143	93	158	184	131	—	—
Degreased and mineral oil added	142	122	137	210	63	—	—
Treated with 5% NaHCO₃	37	136	293	316	—	—	131
Treated with 10% NaHCO₃	0.2	48	258	276	—	—	—

to sole, chrome-retan and hydraulic packing leathers. Sodium bicarbonate undoubtedly exerts its beneficial effect by decreasing the acidity and thus lessening the hydrolytic action. Degreasing was definitely harmful, and this is in accord with other reports that the more ready access of water to the leather fibers favors hydrolysis. Lollar and Steinle (74) stored chrome-vegetable retan leather, with and without the addition of a fungicide, under four different sets of conditions to simulate (1) moderate temperature storage, (2) desert storage, (3) moist storage, and (4) storage at 100 per cent relative humidity. After one year's storage, stitch-tear and ball-burst strength determinations, as well as chemical analyses, were made on the samples. The results of the ball-burst strength test are shown in Table 2. The stitch tear results showed

TABLE 2. EFFECT OF AGING ON THE BALL-BURST STRENGTH* OF
CHROME-VEGETABLE RETAN LEATHER, WITH AND WITHOUT A
FUNGICIDE, TESTED UNDER VARIOUS STORAGE CONDITIONS
[FROM LOLLAR AND STEINLE (74)]

Tanner	Treatment Para-Nitro-phenol	Control (no Storage)	Moderate Temp. Storage for 1 Year	Desert Storage for 1 Year	Moist Storage for 1 Year	Storage at 100% R.H for 10 Months	Totals
A	+	437	504	441	443	318	2143
	−	388	372	365	311	314	1750
V	+	332	311	316	226	166	1351
	−	401	360	381	290	245	1677
I	+	453	376	386	337	279	1831
	−	432	467	446	352	289	1986
S	+	523	459	494	413	397	2286
	−	667	574	599	510	471	2821
D	+	414	420	427	394	306	1961
	−	411	391	419	409	347	1977
TOTALS		4,458	4,234	4,274	3,685	3,132	19,783

*Data are the sums from 6 specimens and are given as pounds of pressure to push a 1/8-in. diameter ball through the leather.

the same general trends. From examination of the data, the leather deteriorated rather badly, especially the samples stored under moist conditions. That damage was more severe than shown by the tests of Kanagy and co-workers (66) can be explained by the longer exposure period or by the higher exposure period or higher exposure temperature.

Although the investigators cited above attribute the loss in strength

to a hydrolytic action—and certainly this is a natural inference, since the conditions that favor this deterioration, i.e., high moisture, high temperature, and low pH, would also favor hydrolysis—no direct evidence has come to the writer's attention that this mechanism is actually involved. Nevertheless, the evidence seems well founded that deterioration of leather does occur irrespective of the presence of mold growth (40). Molds may contribute indirectly by increasing the humidity. Also, the production of acids by these molds might be a significant factor in this type of deterioration.

Relation of Leather Type to Mold Growth

Those who have worked with leather know that vegetable-tanned leather is more susceptible to growth of molds than chrome-tanned leather. Chrome, retanned with vegetable, occupies a position between these two methods as to susceptibility to mold growth (48, 102). The statement by Smith (106) that chrome-tanned leather is almost completely resistant to mold growth is not agreed to by other workers (1, 7, 84, 102, 129). The relative resistance of a leather to mold growth is determined far more by the nature of the materials it contains that can serve as nutrients for the fungi, such as greases, sugars, and vegetable tannins, than upon the nature of the tannage. Waksman (129) and others have attributed the degree of resistance possessed by chrome leather to the fact that it is usually heavily impregnated with oils, waxes, greases, etc., and hence the fibers are not readily wet. Chromium salts are said to possess a slight antiseptic action (1, 129) which may play a role. Chamois leather is also relatively resistant to mold growth (92, 102) although the chamois leather face linings of oxygen masks became quite heavily molded (104). Persons who develop new synthetic tanning materials always hope that the leather they produce will resist the growth of molds. So far, this hope has not been realized. The writer has tested a number of such leathers, and mold has grown on all of them. Day (33, 35) found Orotan leathers to be considerably but not completely resistant to the growth of fungi. Unpublished work by the writer has shown that retannage of vegetable-tanned insole leather with alum imparts a considerable degree of resistance to mold growth.

Fulton, Gibson and Moore (41) were able to demonstrate that vegetable-tanned leather had a fungicidal effect on *Trichophyton mentagrophytes* (*T. gypseum* and *T. interdigitale causative*) agents of "athlete's foot"; whereas chrome-tanned leather had no such effect. They attributed this activity to the vegetable tannins and were able to show a similar effect with tannic acid on the same organisms. Other airborne fungi were not affected by either leather.

Relation of Mold Growth to Environmental Conditions

In regions of the earth where rainfall is abundant and the temperature and humidity high, vegetation grows profusely. It is precisely these conditions that also favor the growth of the scavengers of the plant world, the fungi, which decompose the organic debris and continue the elements in their cycles of use and reuse.

Although mold growth is most profuse in warm, humid climates, these plants actually thrive under a wide variety of conditions. Whereas, most of these filamentous fungi are mesophilic and grow most readily at temperatures of 10° to 37°C (50° to 100°F); some are thermophilic , growing readily at 50°C (122°F); some will also grow at refrigerator temperatures in the range of 2° to 10°C (35° to 50°F). Molds will also tolerate a wide pH range; from pH 2, or even less, to pH 8 or more; the optimum range is about pH 4 to 7.

Fungi are able to attack a great variety of organic compounds, including fats, waxes, simple carbohydrates, proteins, celluloses, lignins, and many other simple and complex organic materials. It can be readily seen that the materials with which leather is treated, such as finishes and stuffing compounds, will play a very important role in determining its susceptibility to mold growth.

One of the most important requirements for mold growth is the proper moisture conditions. A knowledge of the conditions under which leather can be safely stored or transported, particularly on long sea voyages, is a matter of considerable practical importance. Such studies as those of Groom and Panisset (49) and Galloway (42) are often cited for proof of the statement that mold fungi will not germinate and grow below about 70 to 75 per cent relative humidity, and that the humidity at which fungi will grow is determined by the nature of the fungi present and not by the nature of the material. More recent studies have shown that the problem is far more complicated than was supposed. Colin-Russ (20) considers that the most important factor effecting mold growth is the "available moisture," "i.e., the moisture held by the growth and derived from the substratum beyond the normal equilibrium regain at the humidity of the environment considered. Rose and Turner (100) demonstrated that mold growth is dependent on the moisture content of the leather, irrespective of the relative humidity of the ambient atmosphere. This suggests that little moisture is taken up by the molds from the atmosphere but must be obtained from the substrate.

Block (12) made an excellent study of humidity requirements for mold growth on six different organic materials, including leather. He concluded that the more hygroscopic a material is, the lower is the relative humidity at which it would support mold growth. In a 1-year test, shoe

upper leather was susceptible to growth of molds at 76 per cent relative humidity and higher. Block's data appear to indicate that nutrients and inhibitors affect essentially the rate of growth and not the minimum humidity for mold growth. However, in practice, the rate of growth is the controlling factor where the lag phase, i.e., the time for growth to appear, is extended considerably by lack of nutrients or the presence of inhibitors. Thus, a low concentration of a fungicide that is ineffective at a high relative humidity may so delay the appearance of mold at a lower humidity that, for all practical purposes, there is no mold problem. The minimum humidity for the occurrence of mold growth was related to the equilibrium moisture content of each material at each humidity. The minimum moisture contents for mold growth was in the range of 12 ± 2 per cent. In the case of cotton, the only material tested in this way, hygroscopic agents raised its equilibrium moisture content and lowered the relative humidity at which mold growth occurred.

It is thus seen that the water-absorbing properties of a material play an all-important role in determining the limiting humidity of the atmosphere at which mold growth will occur.

ESTIMATION OF FUNGAL RESISTANCE OF LEATHER

Although molds do very little actual damage to leather, their growth on it is highly objectionable, because molds affect associated materials and because moldy articles are repulsive to most people. The time and expense required to recondition moldy material fully justify treatment to prevent mold.

In estimating effectiveness of tests in which inhibitory agents are used on leather, workers should be cautious. A number of very good methods have been worked out for the general testing of inhibitory agents. The American Phytopathological Society (4) utilizes a slide-germination technique in which the spores of a given species of fungus are placed on glass slides that have previously been treated with a spray or dust of the material under test. The slides are then placed in a moist chamber and after a certain incubation period the number of germinated spores are counted with the aid of a microscope. This method is very useful for screening compounds against particular fungi, as for example, those causing plant diseases. Mandels and Siu (77) have adapted a manometric procedure to determine the susceptibility of various materials, including leather, to the growth of molds. Although only 24 to 48 hours are required for the test, some doubt of its reliability for leather has arisen, because in some cases autooxidation of the leather or compound decomposition has resulted in apparent respiration. In a later

paper Mandels and Darby (76) reported an even faster method, which is based on the fact that spores swell to many times their normal size during germination. By measuring the increase in cell volume in the presence and absence of the material under test, the effectiveness of the material for preventing germination is determined. This test requires only 3 hours. The Fungicide Subcommittee of the Deterioration Prevention Committee of the National Research Council has adopted a screening test method (88) for determining the fungicide activity of candidate fungicides in which the compound under test is incorporated in a nutrient agar medium, which is then inoculated with a certain strain of *Aspergillus niger* van Tieghem. The efficiency of the compound is judged by the amount of growth.

Such methods serve a very useful purpose in screening large numbers of candidate compounds, but the methods are of very limited usefulness for final evaluation.

Another approach to the problem is to estimate the amount of deterioration by nondestructive tests. Erich, Larsen and Keck (37), under contract from the Army Quartermaster Corps, investigated electrical conductivity, thermal conductivity, radiation absorption, molybdenum, x-radiation, and dielectric coefficient measurements as related to leather deterioration. Of these only x-radiation was promising. Kanagy and Robinson (65) have studied the sonic technique as a means of measuring leather breakdown. Such methods would be very useful if they could be perfected.

In 1934, Thom, Humfield and Holman (112) published a method for determining the mildew resistance of outdoor cotton fabrics. In this procedure strips of fabric are soaked in water, sterilized, and inoculated with a pure culture of *Chaetomium globosum* Kunze, a cellulose-decomposing fungus. The strips are incubated 14 days on an agar jelly medium containing mineral nutrients. After conditioning, tensile strength is determined. For the purpose intended, this method works very well, but it seems to have exerted undue influence on the methodology of determining the mildew resistance of leather. This method contains a number of features that are not applicable to leather; for instance, the sterilization of the sample, the use of a cellulose-destroying organism for inoculation, and method of evaluating tensile strength measurements.

O'Flaherty and Doherty (94) suggested a method in which a nutrient agar medium is inoculated with molds from moldy leather and the treated leather is placed on the surface of the hardened agar. If, after 24 to 48 hours incubation there is a zone of inhibition around the leather sample, it is considered to be well protected. Although this does indicate inhibition or the growth of the organism, it also shows that the test ma-

terial has diffused out of the leather into the agar and is probably too soluble to be of much value. This method, modified as to the inoculum, is specified by the Canadian Government for use in testing the resistance of leather to athlete's foot organisms (15, 16). In a modification of this method, which has been used by a number of workers (26, 34, 64, 114, 117), spores of *Aspergillus niger* are used to inoculate a nutrient agar medium. Treated leather samples are placed on the surface after the fungus has grown and produced a mat of mycelium (1 or 2 days). The degree of protection is assessed by the occurrence of lack of growth on the leather specimen after a further incubation period of 1 week. On a theoretical basis this method leaves much to be desired (22). However, it seems to give consistent results that can be used to predict, in a general way, the performance of a fungicide under field conditions. This method is one of the three permissible for testing the mildew resistance of leather under Federal Specification KK-L-311a, Method 5021 (127). Another very similar method, 5031, is also included. It employs as the inoculum a spore suspension of the fungi *Myrothecium verrucaria* (Albertini and Schweintz) Ditmar and *Aspergillus ustus* (Bainier) Thom and Church in a water extract of a "soil rich in microbial life." No scientific justification for the use of such an inoculum has been published insofar as the writer is aware. It has several disadvantages. The difficulty of standardizing or replicating a "soil rich in microbial life" and the introduction into the leather of a soil infusion that is known to be harmful, thus adding another variable to the test.

The British Leather Manufacturers Research Association (36) has adopted a procedure in which the treated leather is merely maintained at a temperature of about 30°C and 100 per cent relative humidity for 4 weeks. Inoculation is not considered necessary. In the majority of cases this procedure would probably be satisfactory. However, it is possible that a particular sample might not contain spores of molds that could grow readily upon it. For this reason inoculation is usually considered advisable.

The question as to whether to use a pure culture or mixed cultures has received considerable attention. One reason that pure culture techniques are not more popular is the difficulty of sterilizing leather without damaging or altering it. Several methods have been devised for using heat (91), formaldehyde vapor (90), or methyl alcohol vapor (8) to sterilize leather, but all have disadvantages and none has been accepted. It is not necessary for the leather samples to be sterilized, as the growth of any fungus on the test specimen constitutes failure of the treatment.

In order that a standard method would be available for use by the leather industry, workers at the Tanners' Council Laboratory at the Uni-

versity of Cincinnati and at the Eastern Regional Research Laboratory in Philadelphia collaborated in developing a method (3,22) that has since been adopted as the Official Method of the American Leather Chemists' Association (ALCA). It is also included as a permissible procedure for government goods [Method 5011, Federal Specification KK-L-311a (127)], and the method has found wide acceptance in this country and has been used abroad (13).

The method is very easy to perform, requires no equipment such as sterilizers, etc., and can be carried out by nontechnically trained operators. Essentially, it consists of inoculating the leather specimens with mixed spores of 14 different molds, which are available in a dry form from the University of Cincinnati, and incubating them at a temperature of 30°C and a relative humidity of over 85 per cent. The amount of growth is assessed visually, as no quantitative measure of destruction is possible. The one disadvantage of the method is that a considerable length of time is required for incubation, although if a treatment fails it is frequently known very soon. O'Flaherty (93) regards the *Aspergillus niger* mycelial mat method and the ALCA mixed spore method as valuable complementary tools in evaluating the mold resistance of leather.

Musgrave and Turner (87) have introduced a method for evaluation of fungicides that includes what they term "pretreatment." Leather samples are placed in a warm, humid atmosphere, and mold is allowed to develop. After 49 days the amount of mold growth is recorded and brushed off. The samples are then treated with the fungicidal material and replaced in the humidor and incubated at 30°C. Simple and effective humidors were made by placing small glass tubes (3 x 1½ in.) inside larger tubes (4 x 2 in.); the small tubes carried the leather samples and the large tubes contained water and were closed by rubber stoppers. a comparison of (1) fungicide-treated leather samples, (2) not deliberately infected, (3) infected by dusting with spores, and (4) "pretreated" is given in Table 3. Growth occurred on both "pretreated" and inoculated samples whereas no growth occurred on the uninoculated samples. The long incubation period for pretreatment would be a serious disadvantage for the use of this procedure. Furthermore, any evaluation in a closed system "humidor" is apt to give erroneous results if the compounds are volatile. They remain in the closed system and prevent mold growth, but in actual use the volatile compounds are dissipated into the atmosphere and the protection is lost.

Green (46) has modified the method of Musgrave and Turner (87). Leather samples are incubated over water until profuse mold growth occurs. This permits the selection of leather suitable for the test and

TABLE 3. FUNGICIDAL POTENCY ON LEATHER BY DIFFERENT
TREATMENTS AND DIFFERENT TYPES OF INFECTION
[FROM MUSGRAVE AND TURNER (87)]

	Extent of mold growth on								
Treatment	5th day			15th or 16th day			35th or 36th day		
	Sample No.			Sample No.			Sample No.		
	1	2	3	1	2	3	1	2	3
With approximately 0.2% w/w p-nitrophenol on "pretreated" samples*	0	1	0	3	3	3	9	3	3
Samples deliberately infected by dusting	0	0	0	3	3	2	8	10	0
Samples not deliberately infected	0	0	0	0	0	0	0	0	0
With approximately 0.1% w/w phenyl mercury chloride** on "pre-treated" samples*	0	0	0	1	3	1	7	10	6
Samples deliberately infected by dusting	0	0	0	0	0	0	3	3	0
Samples not deliberately infected	0	0	0	0	0	0	0	0	0

*Leather placed in warm humid atmosphere and mold allowed to develop for 49 days; then mold is brushed off.
**The phenyl mercury chloride was applied in warm neatsfoot oil, as this permitted the application of a greater dose by increasing solubility.

provides moldy leather which is used to inoculate treated specimens. These specimens are incubated at 25° to 30°C in closed containers over 0.4 per cent sodium hydroxide solution. The inoculation is repeated after 14 days. Phenolic fungicides, if volatilized will be absorbed by the alkali and may be analyzed for at the end of the test.

Such a procedure might be justified in special cases but would be too laborious and time consuming for screening tests.

In 1940 Colin-Russ (18) introduced the concept of "the proneness to mold growth" of leather. This term includes character of growth, objectionable odors, ease of removal, weight of growth per gram of air-dry material, and weight of growth per gram of water present in excess of that present at equilibrium with 75 per cent relative humidity. As the determination of all these factors would be laborious, the method has not been accepted. Furthermore, this criterion is not so severe as the mere appearance of growth, which is normally used.

Useful as these laboratory tests are, they cannot give final information as to how a treatment will stand up under field use. A much more rigorous exposure is afforded by use of specially constructed tropical testing chambers (21, 55, 96). In these rooms, the relative humidity and

temperature are automatically cycled so that conditions of the humid tropics may be approximated. Forest litter from tropical plants with its natural flora and fauna to provide inoculation, is kept in the room. It is believed that such a room represents as faithful a simulation of tropical environment as may be obtained in the temperature zone. Dahl and Kaplan (26) have found that laboratory tests are useful only for pre-linimary screening of fungicides and that the only test which may be as-sumed to predict fairly reliably the field performance under tropical jungle conditions is the tropical room exposure test.

Evaluation of materials by testing *invitro*, or in tropical chambers, can be meaningful only if tests are correlated with actual field con-ditions. Therefore, the United States Army set up a number of exposure sites to test leathers under various climatic conditions. Such sites are located at Savannah, Ga.; New Orleans, La.; Fort Lee, Va.; Yuma, Ariz.; Washington, D. C.; Beltsville, Md.; Las Cruces, N. Mex.; Saucier, Miss.; Homestead, Fla.; Big Delta, Ala.; and the Panama Canal Zone (119, 126).

Other countries also maintain such exposure sites. One site that af-fords a wide variety of climatic conditions from tropical rain forest to arid desert is maintained by the British in West Africa (2).

Thus, methods and facilities are available for testing the effective-ness of fungicides for leather that will give an accurate estimation of performance under a wide variety of conditions of use.

CONTROL OF MOLD GROWTH ON LEATHER

General Considerations

Molds are members of a group of plants known as fungi. They differ from green plants in that they cannot utilize carbon dioxide and the energy of sunlight to synthesize their cell constitutents, but must rely on organic carbon and, in some cases, organic nitrogen as well. They must also have a supply of oxygen and, as mentioned above, the con-ditions of moisture and temperature must be within the limits of their endurance in order for the molds to grow. If any of these essential con-ditions are not met, the organism cannot grow. Thus, eliminating the food supply or oxygen or subjecting the molds to unsuitable conditions of temperature or moisture will prevent their growth. However, in most cases, it is not practicable to exclude completely oxygen, and, sur-prisingly, even a small amount of nutrient such as that left by a finger print, will provide food for growth. Also, the material to be protected, or an essential component of it, may serve as the nutrient material. Much can be done to prevent growth by temperature control, however, although molds can tolerate wide ranges in temperature. Under certain

conditions it is possible to prevent growth by reducing the moisture content below 12 per cent by means of desiccants or by raising the temperature. Such physical controls are very useful, but cannot be applied under many conditions of use. By far the most useful means of preventing mold growth on leather is the application of chemical agent.

Requirements of an Acceptable Agent

The chemical agents, which may be fungicides and thus kill the molds or fungistats and merely prevent their growth, must have very specific properties in order to be useful for treatment of leather. Other workers (6, 24, 53, 73, 85, 107, 126) have listed the following requirements for a suitable material:

(1) It must be toxic to a wide range of fungi; in fact, it should prevent the growth of any fungus capable of growing on leather.
(2) It should be nontoxic and nonirritating to human beings, in the concentrations used.
(3) For many purposes it should be colorless or at most, light colored.
(4) It should be odorless; or if there is any odor, this should not be unpleasant.
(5) It should not be volatile.
(6) It should be easy to apply under ordinary commercial conditions.
(7) It should be stable to heat.
(8) It should have no bad effect on any dyeing or other finishing process.
(9) It must not affect adversely the physical properties of the leather.
(10) It must not have any corrosive or other deleterious effect on metals with which the leather may come in contact.
(11) It should be stable to light and should not accelerate the degradation of the treated leather by light.
(12) It should be resistant to leaching.
(13) It must not adversely affect the waterproofness of the leather.
(14) It must not be prohibitively expensive.
(15) It must be available in the quantities required.
(16) Methods must be available for the determination of the amount of the substance present in treated leather.

No material is available that meets completely all the requirements of this formidable list, nor is any apt to be found. Therefore, the use of a chemical agent is always a compromise—the desirable properties balanced against the undesirable. Fortunately, these requirements for a fungicide do not have to be met for all leathers. For instance, resistance to leaching is not important in a leather that is not subjected to such conditions, and toxicity is less of a problem in leathers that do

not come in contact with the skin than those that do. It is also true that all leathers do not require the same degree of protection; leathers to be used in the tropics are much more difficult to protect than those that are used in a temperate climate. For some uses, no protection is needed.

Mold-Inhibiting Agents for Leather

Additives that have been considered for use in leather include almost every material which has been suggested for control of mold growth. Turner, Musgrave and Rose (115) have included in an annotated list most of the compounds tested as leather fungicides. Many of the comparisons, however, were intended either as a means of estimation of the value of a material for control of mold growth in the tannery or as a means of sterilizing worn leather articles such as shoes. Furthermore, there is no uniformity as to methods of test or criterion of effectiveness. As a result, many of the results are of doubtful value for extension to field use. Of the references appearing since 1940 that contain recommendations of material to prevent mold growth on leather, about 40 of them contain no original research but are of a general nature, i.e., government specifications, conference recommendations and reports. About 50 references relate to original works on the subject. Publications by Turner and co-workers (115), Robertson's chapter (99), Musgrave (85) and two compilations of abstracts (54, 130) list materials used before 1940 to prevent mold growth on leather.

Richardson (98) studied the effectiveness of phenol derivatives for preventing mold growth on leather during sammying. Included were the sodium salts of 2,4,6-trichlorophenol; 2,4,5-trichlorophenol; tetrachlorophenol; pentachlorophenol; 2-bromo-4, 6-dichlorophenol; 2-bromo-4-phenylphenol; 2-chloro-4-phenylphenol; o-phenylphenol; and a 4 to 1 mixture of 2-chloro-6-phenylphenol and 2-chloro-4-phenylphenol. Several compounds showed marked fungistatic activity; sodium 2,4,5-trichlorophenate was the best. It prevented growth for 49 days at a 1:5000 concentration. The same group of compounds, but including also p-nitrophenol, were tested for use in controlling growth on chrome-tanned stock "in the blue" (97). Here again, sodium 2,4,5-trichlorophenate was best, but was followed closely by several others.

Maxwell and Lennox (81) tested a group of volatile materials by exposing boots to their vapors for 5 days in closed containers. The effect was transitory, as mold growth appeared 4 days after the boots were removed and exposed at a temperature of 80°F and of relative humidity of 80 per cent. They also tested salicylanilide, sodium pentachlorophenate, sodium trichlorophenate, thymol, and β-naphthol by spraying 0.1 per cent solutions on the surfaces of boots, then drying and exposing

them at 80°F and saturation humidity. In these tests sodium trichloro-
phenate was much more effective than the other compounds tested.

Colin-Russ (18) tested 19 antiseptics and disinfectants. The most
efficient, considering cost, were 0.1 to 0.4 per cent alkaline β-naphthol,
0.1 per cent mercuric chloride and especially, 0.00175 per cent phenyl-
mercuric nitrate. p-Nitrophenol was classed as inferior to β-naphthol.
Effective, but noneconomical were glycocarvolene, p-chloro-m-xylenol,
"Santoxate," and salicylanilide. Later work has not borne out these
results, especially with regard to p-nitrophenol and organic mercury
compounds. This is probably owing in part to differences in the criteria
used to evaluate the materials.

Wade (128) tested dichloro-1:4-naphthoquione, tetraethyl thiuram di-
sulfide, tetramethyl thiuram disulfide, phenylmercuric chloride, phenyl-
mercuric acetate, salicylanilide, and trichlorophenol by dipping case
leather in methylated spirit solutions for 1 minute. After drying, the
samples were divided into three portions: one was washed in a water
spray for 24 hours; another was heated at 75°C for 5 days; and the third
was tested without further treatment. The samples were inoculated with
a mixed spore suspension of Aspergillus niger and Penicillium sp. and
incubated in closed containers over water for 14 days at 30°C. Phenyl-
mercuric chloride and phenylmercuric acetate both gave good results.
Salicylanilide was very effective, and at 1 per cent concentrations no
growth occurred on conditioned or unconditioned samples. Trichloro-
phenol was somewhat less effective. Neither of the thiuram disulfide
compounds showed promise, and dichloro-1:4-naphthoquione was inef-
fective. Results of later tests by other workers would likely show that
salicylanilide would not be so effective if the incubation time had been
extended. Wade also tested terpineol, full strength and in mutton tal-
low, and found it ineffective.

Lollar (72, 73) tested 60 compounds, representing several classes of
commercial preservative agents, and in some classes several members
were included. Leather samples were treated with solutions or emul-
sions of the compounds by drumming for 3 hours. After the leather sam-
ples were dried, they were given a vigorous washing in distilled water
to remove any soluble material. Inoculation was made with a mixture of
molds spores from moldy leather, and incubation was carried out at 85
to 95 per cent relative humidity and at temperatures of 95° to 100°F.
The preservatives that were found effective and the amounts required in
leather for 4 weeks protection are given in Table 4. The relatively large
amounts of organic mercurials required in the tests by Lollar should be
compared to the results obtained by Colin-Russ (19) as well as the ef-
fectiveness of 2, 2'-dihydroxy-5,5'-dichlorodiphenyl methane, (Preventol

TABLE 4. EFFECTIVE PRESERVATIVES FOR LEATHER THAT WERE
EFFECTIVE AGAINST MOLDS AFTER 4 WEEKS AT 95° TO 100°F.
TEMPERATURES AND RELATIVE HUMIDITIES OF 85 TO 95
PER CENT [FROM LOLLAR (73)]

Preservative	Active Ingredients (%)
Preventol I	0.05
Formula 17	0.20
Ethylmercury chloride	0.20
Salicylanilide	0.20
Pentachlorophenol	0.25
p-Chloro-m-xylenol	0.25
2-Mercapto-benzothiazole	0.25
Preventol G. D.	0.30
2,4,5-Trichlorophenol	0.30
Tetrachlorophenol	0.30
p-Nitrophenol	0.30
Phenylmercury acetate	0.50
p-Chloro-m-cresol	0.50

G. D.). Other workers (23, 83) found Preventol G. D. to be practically useless as a fungicide for leather. It should also be pointed out that Preventol I, which is trichlorophenol in triethanolamine, is much too alkaline for use on leather. As a result of this work, Lollar recommended combinations of p-nitrophenol plus pentachlorophenol or salicylanilide plus pentachlorophenol for treatment of leather.

For use in army dubbings, Greene and Lollar (48) tried most of the effective compounds listed above and recommended a mixture of 0.8 per cent p-nitrophenol, 0.8 per cent tetrachlorophenol, and 0.8 per cent p-chloro-m-xylenol or pentachlorophenol mixed in the dubbing compound. With this mixture, chrome-retan army shoe upper leather, grain out, was protected for 9 weeks; and with flesh out, for 5 weeks.

For the sterilization of used army shoes, Greene (47) investigated ethide (1,1-dichloro-1-nitroethane) vapors, methyl bromide vapors, formaldehyde vapors, alkaline hypochlorite solution, and aqueous formaldehyde. A 1 per cent solution of formaldehyde was found to sterilize the shoes in about 5 minutes. The shoes were then washed in a soap solution followed by washing in water or sodium bisulfite, to remove the formaldehyde. Finally they were treated with an oil emulsion containing pentachlorophenol, to give the leather lasting resistance against mold growth.

At the Philadelphia Navy Yard, Grubb and Kime (50) investigated the use of several organic mercurials, zinc naphthenate, salicylanilide, and a dubbing compound containing 3 per cent of a 1 to 5 mixture of 2,4,5-trichlorophenol and 2,3,4,6-tetrachlorophenol for prevention of mold

growth on leather binocular carrying cases. The dubbing compound and two of the organic mercurials were fairly effective, but it is impossible to evaluate the treatments accurately, because no determination was made of the amount of fungicidal material in the leather. For the sterilization of chrome leather infected with *Trichophyton mentagrophytes* (*T. gypseum* and *T. interdigitale*), Fulton, Gibson and Moore (41) found phenylmercuric acetate much more effective than any of the other materials tried. The materials were applied in a fatliquor, and no measure was made of the permanence of the treatments, but only to the initial killing action.

For the prevention of mold growth on bookbindings, Hetherington (52) used the following treating compound: thymol crystals, 10 g; mercuric bichloride, 4 g; ether, 200 cc; and benzene, 400 cc. The compound was applied with a swab held in such a way that none of the material could get on the fingers. No mold appeared on any books so treated. The use of this material would seem to constitute a considerable hazard.

Cordon, Rogers and Mann, in conjunction with the Army Ordnance Department at Frankford Arsenal, developed a treatment for leather carrying cases (120, 121). The treating compound developed after testing pentachlorophenol, *p*-nitrophenol, salicylanilide, and two organic mercurials, contained the following ingredients (in per cent by weight): salicylanilide (shirlan extra), 2.2 ± 0.2; isopropyl alcohol, 25.0 ± 2.0; wax (paraffin), 33.0 ± 2.0; Stoddard solvent, 39.8 ± 5.0.

The Army Ordnance Department would not consider any compound containing halogens or mercury because of possible toxic or corrosive effects. Although salicylanilide was not quite so effective a fungicide as *p*-nitrophenol, it had the advantages of being less toxic and less soluble and was colorless. The paraffin wax was added to impart water resistance and rigidity to the cases, which had paper board as the main structural material, and because salicylanilide was a more effective fungicide when wax was included in the treating compound. This treatment was specified in the Army Ordnance Tentative Specification AXS 1416 (120). Binocular carrying cases treated in accordance with this specification were used in the South Pacific for 10 months by a military scouting group. The treated cases remained in very good condition; there was no mold growth, nor had the leather softened or deteriorated.

The United States Army Signal Corps investigated a number of formulations containing halogenated phenols, organic mercurials, quaternary ammonium salts, *p*-nitrophenol, 2,2'-dihydroxy-5,5'-dichlorodiphenyl methane, and salicylanilide. On the basis of their tests they recommended the use of the AXS 1416 formulation (120), except the wax content was cut to 10 per cent. This was done so that treatment could be made in the field without heating the material to bring it into solution.

At the time the war with Japan ended, the Ordnance Department had issued instructions for the treatment of all leather items with the AXS 1416 formulation (123). It was found later (23, 111) that this treatment, although giving a large measure of protection, would not withstand prolonged exposure (9 months) in the open jungle. However, if a small amount of p-nitrophenol (0.07 per cent) were added, the effectiveness of this formulation was equal to any formulation tried.

At Fort Belvoir, Va., the United States Army Corps of Engineers screened a considerable number of fungicides by the mycelial mat method. On the basis of these tests the corps selected salicylanilide, p-nitrophenol, and pentachlorophenol for further testing (117). These were tested, after application to leather, in the tropical testing room. Formulations found satisfactory were the following:

Type I	% by Weight
p-Nitrophenol	1.5
Pentachlorophenol	1.5
Neatsfoot oil	20.0
Mineral oil	20.0
Cyclohexanone	10.0
Perchlorethylene	47.0

Type II	
p-Nitrophenol	2.0
Neatsfoot oil	20.0
Mineral oil	20.0
Cyclohexanone	10.0
Perchlorethylene	48.0

Type III	
Salicylanilide	2.0
Isopropyl alcohol	25.0
Paraffin wax	33.0
Dry cleaning solvent	40.0

These formulations were incorporated in an Engineer Board specification.

Workers at Massachussetts Institute of Technology (80) tested the Signal Corps formulation (see above) and found leather strips so treated to be completely resistant to mildew attack during the standard mold and water-resistance test. The M. I. T. standard mold test included inoculation with the spores from 10 different molds. They also tested a number of other compounds. Chloro-2-phenylphenol, β-naphthol, and Puratized LN, an organic mercurial applied from solvent solution, gave good results as judged by visual growth on the test samples. When the three

compounds were applied in a dubbing compound only the Puratized LN gave any protection. As the amounts actually applied are not given and the exposure time was short, these results reported by the M. I. T. workers are probably not valid for application to field conditions.

Pendergast (95) tested a group of organic mercurials for controlling mold growth on leather. He found that leather selectively absorbs phenylmercuric compounds from aqueous solutions and that the compounds are not removed by soaking the treated leather in water for 24 hours. Although the leather was in contact with the treating solution for so short a time in these tests that it is unlikely this factor would be operative, longer contact with the solution could well have increased the actual fungicide content of the leather in some of the tests in which very low concentrations were found to be effective. Pendergast claims that the compound added in the proportion of 0.01 per cent of the leather weight seems to moldproof it effectively. This is 5 times more than reported as needed by Colin-Russ (18) and 1/50 as much as Lollor (73) reported as necessary. Differences in the method of testing, time of incubation, and the criterion used to evaluate effectiveness probably accounts for these descrepancies.

Klemme and Baldwin (68) tested 49 compounds for use in dubbing to prevent growth of *T. mentagrophytes* and *Epidermophyton floccosum* (Harz) Langeron and Milochevitch in shoe leather. Eighteen of the 49 showed moderate to excellent inhibition against the test fungi. Four of the 18 chemicals—sodium ethylmercurithiosalicylate in 0.25 and 0.50 per cent concentrations; 3,5-dinitro-*o*-cresol in 5 per cent concentration; *p*-chloro-*m*-cresol, and *p*-chloro-*m*-xylenol, each in a 6 per cent concentration in dubbing—prevented growth of the test fungi on both sides of the leather when brushed only on the finished side of the leather or when brushed on both sides.

Other compounds that, when incorporated in dubbing, inhibited the test fungi on the treated areas of the leather but not on the untreated were: chloride of 4-nitro-5-hydroxy-mercuri-*o*-cresol anhydride (3.0 per cent); phenyl-mercuri-9-acetoxy-12-octadecanoic acid (0.5 and 1.0 per cent); 2,3,4,6-tetrachlorophenol (5.0 per cent); 2,4-dinitro-phenyl thiocyanate (2.0 per cent); *p*-nitrophenol (3.0 per cent); hexylresorcinol (4.0 per cent); and 2,3-dichloronaphthoquinone-1,4 (6.0 per cent). No toxicity tests were made, and it is quite likely some of these compounds are toxic. 3,5-Dinitro-*o*-cresol has been found to lose its effectiveness probably by volatilization (23).

Colin-Russ (19, 20) tested sodium trichlorophenate for use on shoes, but rejected all halogenated phenols because of their odor. After also testing some silico- and borofluorides and phenylmercuric nitrate (which

he considers supreme on a price efficiency basis), Colin-Russ recommended sodium silico or borofluoride or chromium fluoride. However, Kritzinger (71) has found that wet salted hides treated with sodium silicofluoride are more apt to mold than if the compound is not present. Probably the fluoride prevents bacterial growth and thus allows the molds to develop.

During World War II copper 8-quinolinolate was developed by Benignus (10) and has proved to be one of the most effective fungicides available (126). Among those who have reported on its usefulness as a leather fungicide are Yeager (135), Kalberg and Yeager (61, 62) and Day (31). The writer has also found it a very effective fungicide for leather. Its extreme insolubility, while very useful for preventing leaching, makes the material difficult to work with. It has been applied as an emulsion and has also been solubilized. These preparations are quite expensive (11), and probably account for the fact that the leather industry has not accepted copper 8-quinolinolate.

Cordon and co-workers (23) tested about 50 compounds, many of which were newly synthesized, but none of them was effective except the well-known chlorinated phenols and organic mercurials. Other unpublished work included tests with actidione, 4,4'-ethylcyclohexylmethyl pyridine, (Echridine) (17), N-trichloromethylthiotetrahydrophthalimide, SR-406* mercury-*tert*-butylthio-sulphenyl dithiocarbamate, mercury-*tert*-butyl mercaptide, and the phenylmercuric salt of dinaphthyl methane disulfonic acid (Septotan). None of these compounds was particularly effective.

In 1948 Kanagy, Charles and Abrams (63) conducted cooperative tests in which p-nitrophenol, mercaptobenzothiazole, tetrabromo-o-cresol, 2,2'-dihydroxy-5,5'-dichlorodiphenyl methane (G-4), and anilinomethyl-mercaptobenzothiazole were tested. Leathers were treated with formutions containing these compounds and were then tested at the Eastern Regional Research Laboratory, Philadelphia, Pa.; at the Plant Industry Station, Beltsville, Md.; at the University of Cincinnati, Cincinnati, Ohio; and at the National Bureau of Standards, Washington, D.C. Each laboratory group used its preferred test. Results were in fair agreement and showed that p-nitrophenol was the most effective, the 0.35 per cent by weight formulation prevented mold growth under all but the most drastic conditions of leaching. Other compounds tested at the Bureau of Standards were 8-hydroxyquinoline, naphthenic acid terpineol, β-naphthol, dihydroxy-diphenyltrichloroethane, trimethylcetylammonium-pentachlorophenate, zinc silicofluoride, ammonium silicofluoride, magnesium silicofluoride and pentachlorophenol. Only β-naphthol, 8-hydroxyquinoline,

*The present trade name is "Captan."

the silicofluorides, and pentachlorophenol showed any appreciable effectiveness against mildew. The silicofluorides were much too water-soluble to be considered for use on leather, and pentachlorophenol, though more effective even than p-nitrophenol, was considered to be too toxic. p-Nitrophenol was therefore the fungicide of choice.

In December 1945, there was issued "U.S. Army Specification No. 92–61 Compound, Leather Dressing, Preservative, for Field Treatment," which specified p-nitrophenol as the fungicide. Many new compounds were tested during the next 5 years, but none were better than p-nitrophenol. At least this was the belief of the military, for in September 1950, it was announced that after July 1, 1951, all leathers procured by the military departments would be required to contain 0.2 to 0.3 per cent of p-nitrophenol, and Specification MIL-L-10095A was issued (124). This announcement was made in articles that appeared in the leather trade journals, and recommendations for the use and determination of p-nitrophenol were included (67, 108, 109, 110, 113). Later, Lollar and Steinle (74) published information about p-nitrophenol that included properties of the compound, biological activity, use as a leather fungicide, and analysis in leather. The Prevention of Deterioration Center National Research Council has compiled a great deal of data concerning p-nitrophenol (89). The center included data on physical properties, fungicidal efficiency, stability, method of determination, effects on other materials, toxicity, leachability, sunlight stability, methods of application and formulation, and a number of tables that compare p-nitrophenol with a great many other materials for a variety of uses.

Even though p-nitrophenol is now required in all military leathers, it is recognized that it has certain objectionable properties, such as its yellow color and solubility (89), and the search for the ideal leather fungicide goes on.

Fialka and Kibria (39) investigated the use of p-nitrophenol, β-naphthol, BSM 11 (14), cresylic acid, zinc sulfate, borax, and Santobrite (sodium pentachlorophenate) for the prevention of mold growth on sheepskin leather. These materials were applied to the leather both along with the clearing solution (wash) and with the pigment finish. After finishing, the leathers were exposed at 100 per cent relative humidity and observed over a period of 2 weeks. p-Nitrophenol was found to be best, with the 1 per cent strength effectively preventing mold growth. β-Naphthol was not nearly so effective as p-nitrophenol and Santobrite; zinc sulfate, borax, and cresylic acid were practically worthless. Very likely many of these water-soluble materials would be completely lost from the leather. No estimation of the amount left in the leather is possible.

By the use of a new test method already discussed, Musgrave and Turner (87) tested a number of proprietary compounds and several well-known fungicides, including p-nitrophenol, organic mercurials, and chlorinated phenols. They concluded that p-nitrophenol, trichlorophenol, and phenylmercuric chloride were outstandingly good fungicides; the limited solubility of phenylmercuric chloride detracted from its use. β-Naphthol was a good fungicide in practicable doses. Some organic mercurials gave only transitory protection; leathers treated with them did not develop mold growth but, if reinfected after treatment growth did develop.

For the protection of leather gloves, hat sweat bands, helmet liners, flying boots, instrument cases, and similar gear, Townsend (113), working at the Wright Air Development Center, tested o-phenylphenol, p-nitrophenol, 2,2'-dihydroxy-5,5'-dichlorodiphenyl methane, p-chloro-m-xylenol, and trichlorophenyl acetate. On the basis of mildew resistance and metal corrosion tests, it was recommended that leather containing 1.6 per cent o-phenylphenol, compounded in a vegetable oil, be considered satisfactory for United States Air Force use in items involving intimate, prolonged skin contact, although clearance from the air surgeon had presumably not been obtained. In a much more extensive report Townsend and Albert (114) gave the results of test of 42 experimental formulations containing fungicidal chemicals as protective treatments against mildew on leather. Twenty-one formulations contained o-phenylphenol, 11 contained fluorinated compounds, 3 contained trichlorophenyl acetate, 3 contained p-nitrophenol, 2 contained p-chloro-m-xylenol, and 2 contained di-lauryl dimethyl ammonium bromide and 2,2'-dihydroxy-5,5'-dichlorodiphenyl methane as the active ingredients. To determine the fungistatic effectiveness of each treatment, at least one of two methods was employed: the A. niger mycelial mat or the A.L.C.A. mixed-spore method.

All treatments except four inhibited the growth of fungi to some degree; the formulations containing p-chloro-m-xylenol, 2,2'-dihydroxy-5,5'-dichlorodiphenyl methane, and di-lauryldimethyl ammonium bromide were ineffective. Although p-nitrophenol was found to be much more effective than o-phenylphenol at the same concentration, p-nitrophenol was not recommended where intimate contact with the skin is involved, because of its toxicity to human beings. This decision was reached despite the fact that the surgeon general of the army had cleared p-nitrophenol for use in boots and the compound was not considered a toxicity hazard when used at the required concentrations. o-Phenylphenol was considered satisfactory for leathers touching the skin, and a method of analysis was developed for determining its concentration in leather. Fluorine compounds were fungicidal at much lower cencentrations than o-phenylphenol, but more work would be required to develop an applica-

ble formulation for use as a leather preservative. As a result of these studies the United States Army Air Force issued a specification for "Leather Dressing, Mildew-Preventative," MIL-L-8067 (USAF) Nov. 4, 1952, which was superseded on May 18, 1955, by MIL-L-8067A (USAF) (125). This specification calls for o-phenylphenol as the fungicide.

For the protection of perishable materials, including leather goods, from mildew during storage, Shumard (105) tested 8-quinolinol, 2,4,5-trichlorophenol, o-benzyl-p-chlorophenol, and p-dichlorobenzene. Under the conditions of the test, in sealed jars, all the materials prevented mold growth. p-Dichlorobenzene was selected for further study on the basis of effectiveness, convenience, relative nontoxicity, commercial availability, rapid dissipation of odor, and public acquaintance. In storage tests this chemical was found to prevent mold growth on a variety of materials, including leather shoes. Because of rapid volatilization, p-dichlorobenzene could only be used in sealed containers and would have no lasting effect.

Vegetable-tanned leather treated with various fungicides was exposed in a humidity cabinet by Grassman and Stadler (43). If a fungicide is judged by the minimum concentration, based on leather weight, that completely prevents fungus attack, the most effective compound was an organic mercury preparation, Sch 101(1:40,000). Next best compounds and effective in 1:7000 dilutions were p-chloro-m-cresol (Raschit K), Preventol (CMK), 1,3,5-trichlorophenol (pure and in Preventol liquid 1) pentachlorophenol, and o-phenylphenol (Preventol O). Raschitol (50 per cent effective phenolic compound content) was effective in 1:5750 dilution. Moderately effective compounds (1:3500 dilution) included KM 11 (a formulation based on p-chloro-m-cresol), dichloro-m-cresol, symetrical dichloro- and p-chloro-xylenols, and p-nitrophenol. Bifluorides, fluorides, fluorosilicates, formaldehyde, hexamethylenetetramine, corthymol, and nipacombin (ester of p-oxybenzoic acid) were ineffective. The low effectiveness (1:350) of Novex (2,2'-dihydroxy-5,5'-dichlorodiphenylsulfide) and of 8-quinolinol on leather samples was striking when compared with the effective dilution in agar tests.

Hausam (51) studied the effect of chlorine substitution in phenol on the effectiveness of these compounds as leather preservatives. He found that dichloro derivatives are generally more effective than either the mono or trichloro compounds, and that a chlorine atom is more effective in the para position than in the ortho position. The number of methyl substitutions also has some influence on the inhibition of fungi; the effectiveness increased directly with the number of methyl groups. Raschitol, a combination of chlorinated phenols was the most effective of the materials tested. It was followed closely by 2,4-dichloro-m-cresol

and to a lesser extent, by 2,4-dichloro-3,5-xylenol. In a 16-day exposure test to *Penicillium* sp., Raschitol was effective in 1:12,000 concentration, followed by 2,4-dichloro-*m*-cresol, 4-chloro-*m*-cresol, 2,4,6-trichlorophenol, and *o*-phenylphenol. All other products were less effective. Pentachlorophenol and *p*-nitrophenol were very disappointing.

According to Anders (5) mold growth on leather and fabric transmission belts can be entirely prevented by the use of a "Raschit" preparation. The active ingredient of these compositions is *p*-chloro-*m*-cresol or its sodium salt. Raschit K may be applied to leather belts as a 0.2 per cent solution in leather oils; Raschit W is used for textile conveyor belts as a 0.2 per cent aqueous solution. These preparations have no adverse effects on leather or textiles. Jamet (58) found that *p*-nitrophenol, *p*-chloro-*m*-cresol, and sodium pentachlorophenate gave satisfactory protection to bookbinding leathers. Sodium potassium tartrate, sodium lactate, or potassium lactate increased the durability of the bookbinding leather but these substances encouraged mold growth.

That the Office of the Army Quartermaster General still is not completely satisfied with *p*-nitrophenol as the leather fungicide is attested to by the fact that they are still supporting research to find better ones. Also, it is highly desirable to have more than one suitable fungicide approved in case one was not available in an emergency. Dahl (24) and Dahl and Kaplan (26) screened a number of chemical compounds by the *Aspergillus niger* mycelial mat method or by the American Leather Chemists' Association mixed-spore suspension method. Materials that prevented mold growth or inhibited it to a considerable extent were further tested by the tropical room exposure test. In many cases a treatment that made the test leather mildew-proof in the initial screening test failed even to inhibit mold growth in the tropical room exposure test. Eight of the more promising fungicides found in the screening program were included in a field exposure study. *p*-Nitrophenol was included as a comparison standard. The other seven chemicals were 4-thiocyanophenol, *o*-chloro-*p*-nitrophenol, bis (4-nitrophenyl) carbonate, tetrachlorohydroquinone, N-trichloromethylthiotetrahydrophthalimide, *o*-phenylphenol, and *p*-chloro-*m*-xylenol. The latter two compounds were included, even though they had been found comparatively ineffective in the tropical room screening test. This was done to permit comparison of field exposure results and screening tests results on some relatively ineffective fungicides as well as on relatively effective ones.

Three levels of concentration of each fungicide were applied to three different types of leather. The concentrations used were the "borderline concentration," i.e., the minimum concentration of the fungicide in the leather to make it mildew-resistant under the tropical room conditions,

one-half, and twice that amount. The leathers used were a vegetable-tanned sole leather crust, vegetable-tanned strap leather, and chrome-retanned military-type upper leather.

After treatment the leather samples were divided into four sets. Sample set 1 was exposed at Yuma, Ariz., where the environment was hot and dry. Sample set 2 was exposed on a roof at the National Bureau of Standards, Washington, D. C., to give a moderate, hot dry environment. Both of these lots were protected from the rain. After three months' exposure to weathering the two samples were tested for mildew resistance, together with sample set 3, which had not been exposed to weathering, by exposure in the tropical room at Fort Belvoir, Va. The fourth set of samples were exposed in the jungle of Panama for a period of 12 weeks. Sample set 4 was protected from direct sunlight and rain but was otherwise exposed to an ambient environment at a site located in the jungle on the Atlantic side of the Isthmus of Panama. A summary of the results is given in Figure 1.

On the basis of these results o-chloro-p-nitrophenol which required only 0.15 per cent to make all samples mildew-resistant was rated slightly more effective than p-nitrophenol and 4-thiocyanophenol, which required twice that amount. Bis(4-nitrophenyl) carbonate was not quite so effective, as over 0.3 per cent strength was required to prevent growth on some of the weathered samples. Some of the other materials gave erratic performances and others were quite ineffective. It was concluded that the tropical room exposure test predicts quite correctly the performance of fungicides under severe mold growing conditions. The mycelial mat test and the A.L.C.A. test are useful only for screening. A fungicide failing these tests can safely be rejected, but a treatment that passes will not necessarily be satisfactory under severe exposure conditions.

Kowalik and Sadurska (70) tested p-nitrophenol, o-phenylphenol, p-chloro-m-cresol, and phenylmercuric acetate for fungus-proofing leather bookbindings. Fungicidal concentrations were 0.25 per cent, 0.3 per cent, 0.25 per cent, and 0.005 per cent, respectively. It was found preferable to apply the fungicide before treating the leather with dressing.

Ross and Berk (101) tested 30 fungitoxic agents for their ability to prevent mold growth on vegetable-tanned leather. The American Leather Chemists' Association test (3) was used to evaluate the effectiveness. Water leaching and heat stability tests were also conducted. The results are summarized in Figure 2. Five phenolic compounds were equal to or more effective than p-nitrophenol. These included two dinitrophenol compounds (2,4-dinitrophenol and 2-isopropyl-4,6-dinitrophenol) that are precluded from use because of their high order of toxicity to human
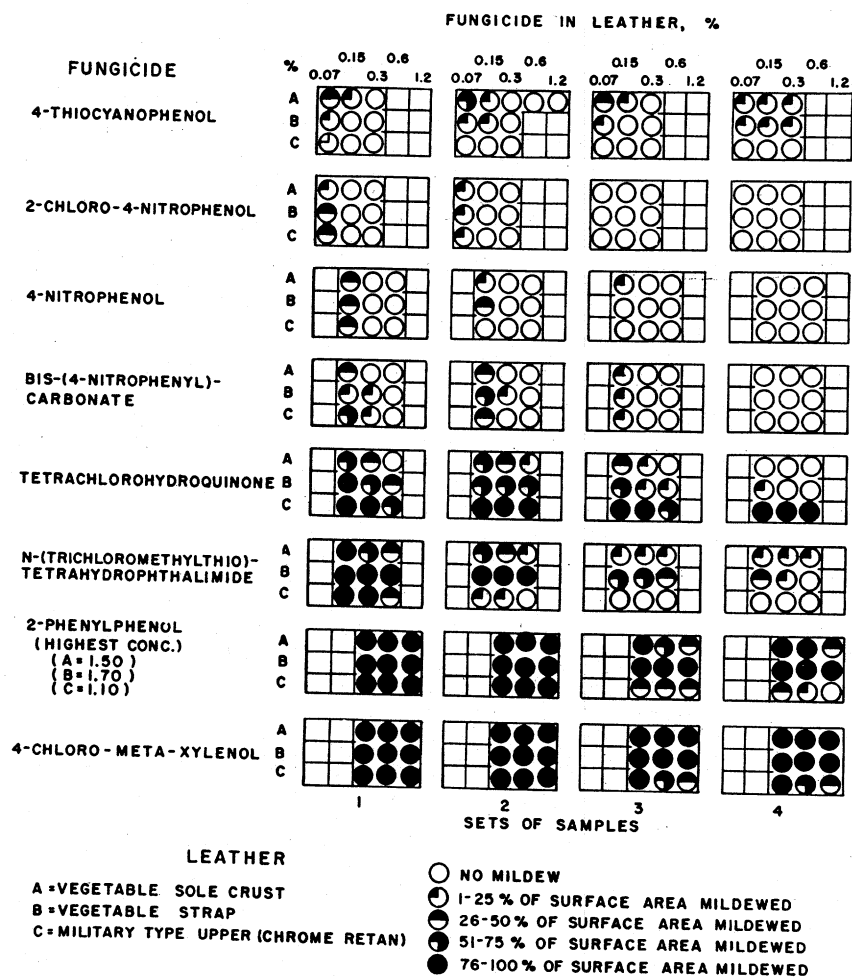
Figure 1. Final mildew growth on leather after completion of mildew resistance tests: (1) Samples 1 were exposed to weathering at Yuma, Ariz., (2) Samples 2 were exposed to weathering on the roof of the National Bureau of Standards Building; (3) Samples 3 were not exposed to weathering but were stored in the NBS laboratory and were tested for mildew resistance by exposure in a tropical room; (4) Samples 4 were not exposed to weathering prior to being tested for mildew resistance by exposure at a Panama jungle site (From Dahl and Kaplan (26).

beings. The others were o-chloro-p-nitrophenol, bis(4-nitrophenyl) carbonate, and bis(2-chloro-4-nitrophenyl) carbonate. The two quinolinolate compounds investigated were found to be effective even after the leaching and heat-stability tests. Ross and Berk concluded that halogenated

p-nitrophenols, including fluorinated derivatives, as well as derivatives based on the bis (phenyl carbonate) molecule, should be further investigated.

Jansing and Roddy (59) have evaluated four aromatic fluorine compounds as fungicides for vegetable- and chrome-tanned leathers. The chemicals tested were 3,3'-difluoro-4,4'-dihydroxybiphenyl; 5,5'-difluoro-2,2'-dihydroxybiphenyl; bis (-2-hydroxy-5-fluorophenyl) sulfide; and 1-fluoro-3-methyl-4,6-dinitrobenzene. Each chemical was formulated with four different finishing oils and applied to vegetable and chrome leathers in concentrations of 0.5 ± 0.15 per cent, 1.0 ± 0.2 per cent, and 1.5 ± 0.25 per cent of the particular chemical being used. Effectiveness was evaluated by performing tests according to Specification KK-L-311a, Methods 5011 and 5021 (127). Treatments with 1-fluoro-3-methyl-4,6-dinitrobenzene gave complete protection to both vegetable- and chrome-tanned leathers at all levels of concentration tested. Treatments with
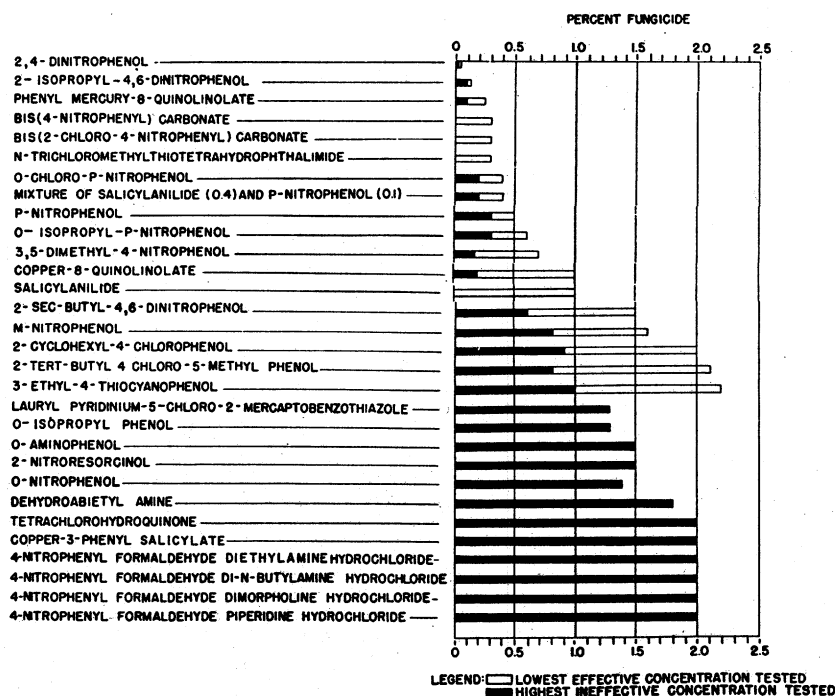

Figure 2. Lowest effective and highest ineffective concentrations of fungicides tested as leather protectants. The treated leathers were leached for 24 hours and incubated for 12 weeks. The minimum effective concentration is within the white bar. (From Ross and Berk (97).

5,5'-difluoro-2,2'-dihydroxybiphenyl gave protection to vegetable-tanned leather at a level of 0.5 ± 0.15 per cent concentration, and to chrome-tanned leather at 1.0 ± 0.20 per cent concentration. Bis (-2-hydroxy-5-fluorophenyl) sulfide and 3,3'-difluoro-4,4'-dihydroxybiphenyl gave about the same protection. Complete protection from molds on vegetable-tanned leather was obtained when a 1.0 ± 0.20 per cent concentration of the above listed compounds was applied, but 1.5 ± 0.25 per cent concentration was necessary to protect chrome-tanned leather. With the exception of 1-fluoro-3-methyl-4,6-dinitrobenzene, these compounds, in the manner and concentrations used, are nontoxic and nonsensitizing to human beings under conditions involving prolonged skin contact. Although performance under field conditions and corrosive properties have not been determined, it appears that the three nontoxic chemicals would merit further study.

A compilation of the test results on 126 compounds that have been screened as leather fungicides has been published by Dahl and Kaplan (27). The initial screening was carried out by using Methods 5021 and 5011 of Federal Specification KK-L-311a (127). This was followed by exposure in a tropical room. Visual examination of the amount of mildew growth on leathers containing known amounts of fungicides was used as a measure of fungicidal effectiveness. Only 6 compounds were as effective as p-nitrophenol. These were: bis (2-chloro-4-nitrophenyl) carbonate; 2,3-dichloro-1,4-naphthoquinone; 2-chloro-p-nitrophenol; 4,6-dinitro-2-methylphenol; 4,6-dinitro-3-methylphenol; and 2,4-dinitrophenyl thiocyanate. These compounds were not evaluated for any of the other important characteristics required of a leather fungicide. Cordon and co-workers (23), however, found 2,3-dichloro-1,4-naphthoquinone to be ineffective when applied in a leather dressing. The vehicle used by Dahl and Kaplan was xylene.

Dahl and Kaplan also investigated two series of compounds structually related to tetrachlorohydroquinone and p-nitrophenol, using a tropical room exposure (28). Effective mildew prevention was associated with 1,4—OH, —NO₂ combinations in 10 compounds, all having a 2,3,5,6,-tetra-chlorobenzene ring in common. Replacement of —NO₂ in this combination with —OH, —CL, and —H decreased effectiveness in that order. Mildew-preventive activity was also associated with 1,4—OH, —NO₂ combination in 11 aromatic nitro compounds without the tetrachloro structure. The effectiveness of bis (4-nitrophenyl) carbonate and bis (2-chloro-4-nitrophenyl) carbonate was found to be associated with their decomposition into free phenols under hot humid conditions.

Additional esters of 4-nitrophenol and 2-chloro-4-nitrophenol were investigated as fungicides for leather by Dahl and Kaplan (29). The

esters tested were 4-nitrophenyl acetate, 4-nitrophenyl propionate, 4-nitrophenyl chloroacetate, 2-chloro-4-nitrophenyl chloroacetate, ethyl 4-nitrophenyl carbonate, 2-chloro-4-nitrophenyl ethyl carbonate, 4-nitrophenyl phenyl carbonate and 2-chloro-4-nitrophenyl phenyl carbonate. All of these compounds were shown to exhibit fungitoxic activity through the liberation of free nitrophenol by hydrolysis. The noncarbonate esters and mixed carbonates, like the bis carbonates, are colorless, less water soluble than the free nitrophenols, and probably nontoxic. Until the free nitrophenols are liberated by hydrolysis, these materials do not have the objectionable features of color and water solubility. However, under warm, humid conditions, when protection is most needed, free nitrophenol is released by hydrolysis. Since this reaction is accelerated by the same conditions that favor mildew attack, a mechanism for the timely conversion of nontoxic to fungitoxic material is provided. Further work along these lines to find compounds of optimum hydrolytic stability and permanence appears to be a promising approach to the leather fungicide problem.

Another promising fungicide for leather, investigated by Dahl and Kaplan (30), is 5,6-dichloro-2-benzoxazolinone. This compound is stable, colorless, nonvolatile, and relatively resistant to removal from leather by leaching with water. The chlorine substituents of the molecule are important for the fungitoxic action since this chlorinated derivative is much more effective than 2-benzoxazolinone. About 0.4 per cent of 5,6-dichloro-2-benzoxazolinone protected vegetable-tanned sole leather from mold growth in tropical room tests whereas approximately 0.2 per cent was sufficient to protect chrome-tanned leather.

The effects of fungicides on the deterioration of leather have been studied by Dahl (25). Under accelerated aging conditions, organically bound copper, chlorine, or chloride ions are less harmful to leather than copper or irons ions. There were some indications, however, that fungicides containing organically bound copper or chlorine may not be completely harmless. In spite of some possible damage to leather by some fungicides, the data presented showed that all the fungicides studied caused less damage than that incurred by the untreated controls. Therefore, there must have been a preservative effect on the leather. This problem of possible damage to leather by fungicidal treatments must certainly be considered.

CONCLUSIONS

At present, serviceable leather fungicides are available that will prevent mold growth. For one reason or another none of them is completely

satisfac'ory. Toxicity, corrosiveness, lack of permanence, and difficulty of use are some of the factors that rule out the use of some otherwise effective materials. Although the Army Quartermasters Corps specifies certain concentrations of p-nitrophenol in the leather they purchase and the United States Army Air Force specifies o-phenylphenol, it must be remembered that this leather must remain free of mold growth under very severe conditions of exposure. Undoubtedly, under normal conditions of use, there are many other compounds that would give adequate protection and that would not have some of the objections of these fungicides.

Many new compounds are being synthesized and tested and it is to be hoped that a more nearly ideal compound will be found.

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